PIP1 Plasma Membrane Aquaporins in Tobacco: From Cellular Effects to Function in Plants

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The molecular functions of several aquaporins are well characterized (e.g., by analysis of aquaporin-expressing *Xenopus* oocytes). However, their significance in the physiology of water transport in multicellular organisms remains uncertain. The tobacco plasma membrane aquaporin NtAQP1 was used to elucidate this issue. By comparing antisense plants that were inhibited in NtAQP1 expression with control plants, we found evidence for NtAQP1 function in cellular and whole-plant water relations. The consequences of a decrease in cellular water permeability were determined by measurement of transpiration rate and stem and leaf water potential as well as growth experiments under extreme soil water depletion. Plants impaired in NtAQP1 expression showed reduced root hydraulic conductivity and lower water stress resistance. In conclusion, our results emphasize the importance of symplastic aquaporin-mediated water transport in whole-plant water relations.

INTRODUCTION

The structural analysis of aquaporins has revealed general mechanisms of protein-mediated membrane water transport (Murata et al., 2000; De Groot and Grubmuller, 2001; Sui et al., 2001), but the physiological functions of this protein class often remain obscure. In some cases, a significant role is indicated by phenotypes of mutant bacteria or animals, symptoms of human diseases, or morphological changes in aquaporin-deficient plants (Agre, 1998; King et al., 2000; Tsubota et al., 2001). Many organisms lacking a specific aquaporin have an unobtrusive phenotype, which suggests that aquaporins are unimportant or can be substituted by homolog proteins or compensatory mechanisms. Hence, the discussion about the role of aquaporins in the majority of multicellular organisms remains open (Chrispeels and Maurel, 1994).

In previous work with Arabidopsis, we demonstrated an increase in plant cellular membrane water permeability by the aquaporin PIP1b (Kaldenhoff et al., 1998). Using a different technique, Barrieu et al. (2000) essentially came to the same conclusion for PIP2b. However, determining the impact of plasma membrane–located aquaporins for whole plant or organ water transport efficiency proved difficult in this model plant because of its small size.

Therefore, our studies focused on a homologous aquaporin of the relatively larger tobacco plant. Accordingly, NtAQP1,

which belongs to a family homologous with Arabidopsis PIP1, was isolated and characterized as a plasma membrane intrinsic aquaporin (Biela et al., 1999). Using RNA gel blot and whole mount hybridization, Otto and Kaldenhoff (2000) found NtAQP1 expression in almost all organs of tobacco, with the highest levels in the root. In situ immunological studies indicated NtAQP1 protein accumulation in the root exodermis and endodermis, in the cortex, close to the vascular bundles, in the xylem parenchyma, and in cells of the stomatal cavities. The aquaporin was found at sites of anticipated high water fluxes from and to the apoplast or symplast.

Here, we describe the characteristics of tobacco plants impaired in NtAQP1 expression as a result of the activity of an antisense construct. These data could lead to a better understanding of aquaporin function not only at the cellular level but also in the whole plant. In addition, an open question of plant physiology, whether the major pathway of water transport in plants is apoplastic or symplastic, could be answered by our studies.

RESULTS

Consequences of Antisense NtAQP1 on Aquaporin Expression

We chose the well-characterized tobacco plasma membrane–located aquaporin NtAQP1 for these studies. This

protein is expressed in virtually all parts of tobacco close to the vascular bundle, with the highest levels in the root; therefore, it is predicted that NtAQP1 most likely contributes to water uptake processes (Otto and Kaldenhoff, 2000). Tobacco plants impaired in NtAQP1 expression were generated by leaf disc transformation with an antisense NtAQP1 construct driven by the strong constitutive 35S promoter of *Cauliflower mosaic virus* together with the kanamycin resistance marker gene NPT II. Regenerated plants were self-pollinated, and the resulting T2 generations of four independently transformed lines were analyzed.

RNA gel blot signal intensities clearly revealed severe reduction of NtAQP1 mRNA steady state levels (Figure 1A) in every one of the antisense lines. To analyze the effect of antisense NtAQP1 expression on RNA levels of other related genes, gene-specific probes for the tobacco NtPIP1a, NtPIP2a, and NtTIPa homologs were generated based on available sequence information. The nucleic acid sequence identity with NtAQP1 was 78, 57, and 38%, respectively. The main difference between NtAQP1 and NtPIP1a was found in the region coding for the protein's N terminus in front of the first of six transmembrane helices. Thus, in contrast to NtPIP2a and NtTIPa, the identity of the remaining major part was very high (>95%).
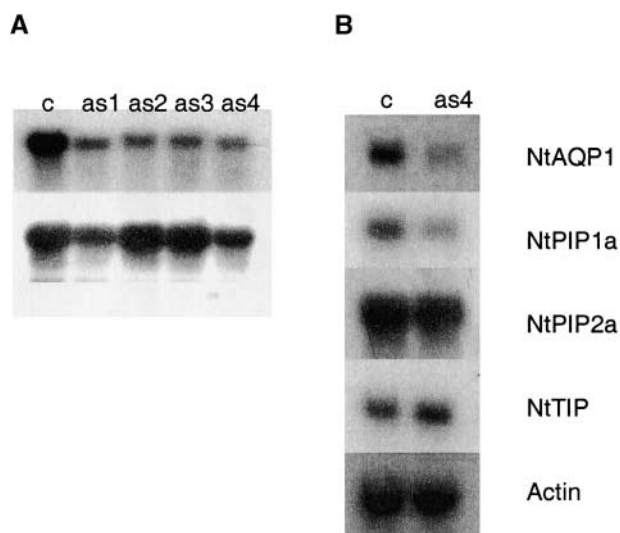

Figure 1. RNA Gel Blot Hybridization Signals with RNA from Control or Antisense Plants.

(A) Hybridization with a probe specific to NtAQP1 (top) or to 28S rRNA as a loading control (bottom). Root RNA was extracted from control plants (c) or from four independently transformed antisense NtAQP1–expressing lines (as1 to as4).

(B) Hybridization with RNA from antisense line 4 (as4) or control (c). Radioactive probes comprising gene-specific regions were synthesized from tobacco aquaporins, which were named according to their Arabidopsis counterparts, NtPIP1a, NtPIP2a, and NtTIPa. As a control for equal lane loading, the complete actin cDNA was used as a probe.

As a representative example, hybridization signals with RNA obtained from line 4 are shown in Figure 1B. Regarding the signal intensities, reduced expression of the closely related NtPIP1a gene is visible, although the reduction was less severe than that of NtAQP1 mRNA, the antisense target. In contrast, RNA levels of other aquaporin genes belonging to other subfamilies were unaffected by antisense NtAQP1 expression. Thus, the results obtained by subsequent analysis of the antisense plants using plant physiology techniques could be correlated to the function of NtAQP1 and closely related PIP1 genes.

Consequences of Antisense NtAQP1 Expression at the Cellular Level

The contribution of NtAQP1 and closely related aquaporins to cellular water permeability was demonstrated by a protoplast-swelling assay. Because NtAQP1 expression was highest in roots and the effect was expected to be most evident in cells with abundant NtAQP1 RNA levels, root cells were chosen for the membrane water permeability studies. Protoplasts obtained from root material were fixed on a poly-L-Lys–covered microscope slide, and the attached cells were kept in a continuous stream of buffer solution. After changing from isoosmotic (300 mosmol) to hypoosmotic (180 mosmol) conditions, the volume change of individual protoplasts was calculated from the increase in protoplast cross-section area. To monitor the velocity of buffer exchange, buffer aliquots were taken directly from the chamber, and the sample osmolarity was determined.

Figure 2A shows average buffer dilution kinetics of 10 experiments and averaged swelling curves of all investigated protoplasts from antisense NtAQP1 or control plants ($n = 36$). Protoplast volume increase started almost instantaneously after the onset of buffer solution exchange (Figure 2A). Because only some of the cells continued swelling for >60 sec and many were bursting, only 1-min data are presented. In addition, experiments that extended the assay with an additional change from hypoosmotic back to isoosmotic conditions revealed a relaxation of protoplasts to their initial volume, which indicates that the protoplasts behave as true osmometers. Accordingly, the chamber is suitable for calculating osmotic water permeability (P_{os}) values of plant protoplasts, taking the initial swelling rate into account. A difference in volume increase rates between cells from antisense and control lines is quite obvious. The average calculated P_{os} for control protoplasts was 27.12 ± 1.8 μm/sec, and the average calculated P_{os} for protoplasts from the antisense lines was 12.35 ± 1.1 μm/sec.

For a more detailed picture, data from individual protoplasts that relate to a linear volume increase were selected for the water permeability calculations. As expected, individual tobacco root protoplasts from both control and antisense lines displayed a wide range of P_{os} values, from 2 to >60 μm/sec. A comparison of the cell populations from the different lines revealed obvious dissimilarities. In Figure 2B,
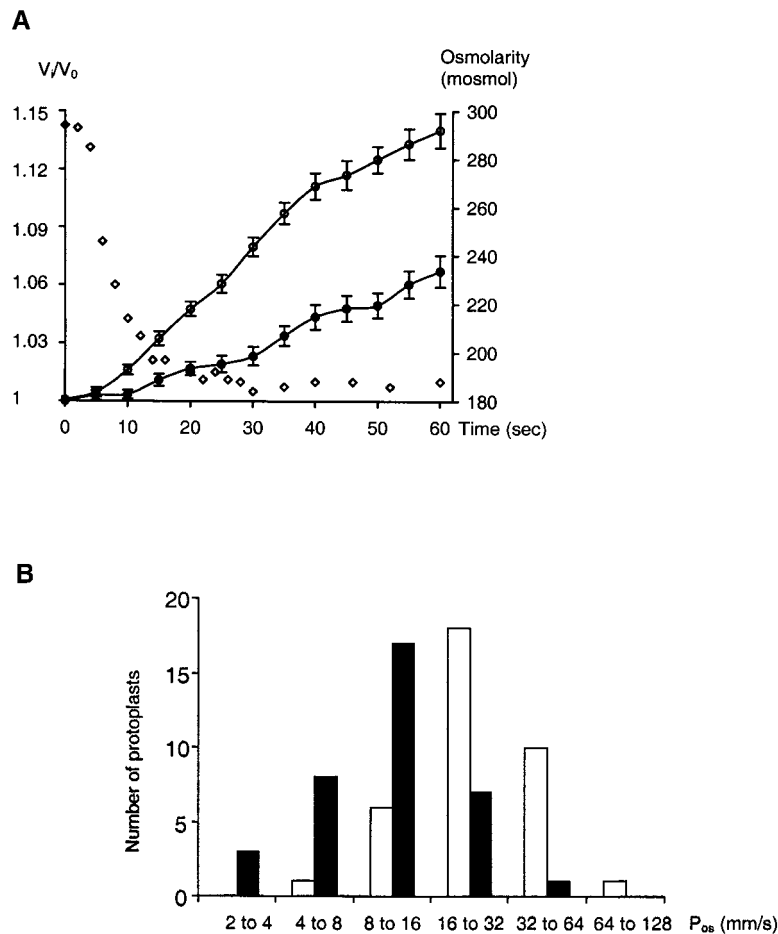
A

B

Figure 2. Protoplast Swelling Analysis.

(A) Protoplasts were produced from roots of control plants (open circles) and antisense NtAQP1–expressing lines (closed circles) and subjected to hypoosmotic conditions. The mean volume increase of control protoplasts ($n = 36$) or antisense protoplasts ($n = 36$; $n = 9$ for each line) in the first 60 sec after osmolarity change is shown. In separate experiments with the same conditions, the osmolarity of aliquots taken from the chamber buffer solution was determined (diamonds; $n = 10$). V_i/V_0, volume increase/initial volume.
(B) P_{os} value distribution of the investigated protoplasts obtained from control lines (white bars) and antisense lines (black bars).

the data obtained from individual protoplasts are collected into groups according to their P_{os} values. Although the protoplast population from control lines peaked in the class with a P_{os} of 16 to 32 μm/sec, the majority of antisense protoplasts showed P_{os} values between 8 and 16 μm/sec (Figure 2B). Therefore, the inhibition of NtAQP1 by antisense expression decreased the overall cellular P_{os}. Conclusively, NtAQP1 function increases the membrane water permeability of tobacco root protoplasts.

Significance of NtAQP1 for Whole-Plant Water Transport

Macroscopic and microscopic inspection of the antisense plants revealed no differences in plant organ morphology in the sizes of cells from root, stem, and leaves or in plant growth. In spite of this finding, occasional slight variations in plant size and root mass were found, but no obvious correlation to growth conditions could be established.

Because NtAQP1 is expressed primarily in roots, it was assumed that a lack of the aquaporin would have an effect on root water permeability. Consequently, root hydraulic conductivity per unit of root surface area (K_{RA}) was measured using the high-pressure flow meter method (Tyree et al., 1995). The K_{RA} of roots from the antisense lines was ~42% of control values ($P < 0.001$) (Figure 3). This decrease was on the same order of magnitude as the mean cellular water permeability reduction. These results indicate that aquaporin expression is essential in maintaining natural root hydraulic conductance. Furthermore, this observation
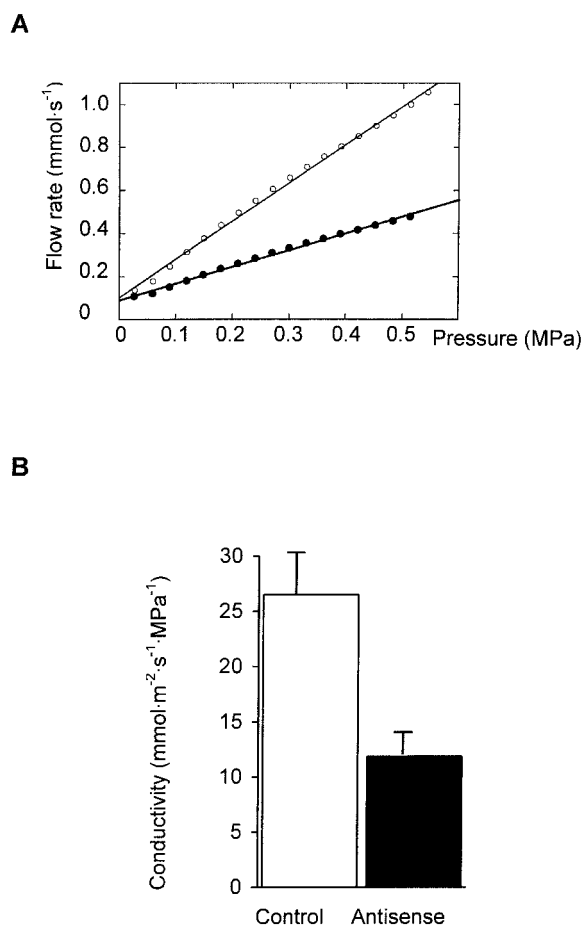
A

B

Figure 3. Determination of Root Hydraulic Conductivity by the High-Pressure Flowmeter Method.

(A) Representative pressure/flow rate relationship of a control plant (open circles) and an antisense NtAQP1 line (closed circles). Both plants had similar root surface areas (control, 0.0605 m^2; antisense, 0.0615 m^2). The applied pressure was increased at 2-sec intervals.

(B) Average specific root hydraulic conductivity of controls and antisense NtAQP1–expressing tobacco plants as measured by the high-pressure flowmeter method. Complete data are given in Table 1.

represents definitive proof that the pathway of water uptake from the root surface to the xylem involves passage across membranes. If the pathways of water transport were completely apoplastic via the cell wall space, there would have been no difference between the K_{RA} of control and antisense plants.

This notion was supported by the determination of K_{RA} before and after two freeze–thaw cycles, which cause mechanical perforation of the cell membranes by water crys-

tals. The root hydraulic conductivities were significantly different (P < 0.003) before the freeze–thaw treatment (55% difference) (Table 1), but they were almost identical afterward (4% difference). This finding also confirms the idea that the function and morphology of the vascular system are very similar in control and antisense plants. An adaptation at the morphological level attributable to the constant low cellular water permeability caused by the antisense effect was not observed.

Nevertheless, tobacco plants appear to cope with the effects of strongly reduced NtAQP1 expression, which might result from optimal growth conditions in the greenhouse with regard to water supply and humidity. However, in a water-limited environment, differences in water permeability could cause a more pronounced effect. Consequently, the two different plant types were not watered for 1 week. During this time, soil water potential decreased from −0.01 to −0.07 MPa, and soil moisture decreased from 62.2 ± 1.63% to 22.1 ± 2.91% (w/w). The most sensitive plant reaction to water stress can be observed by determination of stomatal closure, which is indicated by the transpiration rate. Before and after the drought period, transpiration rate was determined by gas-exchange measurements. In addition, stem water potential (Ψ_{stem}) and leaf water potential (Ψ_{leaf}) were measured by the pressure bomb method on bagged and transpiring leaves, respectively.

The values for transpiration rate, Ψ_{stem}, and Ψ_{leaf} were dissimilar in well-watered conditions, indicating that the absence of NtAQP1 caused stomatal closure and induced a water stress signal, which most likely was induced by the lower hydraulic conductance of the roots in antisense plants. Ψ_{stem} and Ψ_{leaf} decreased in the water stress conditions in both plant lines. However, it became evident that antisense plants remained at more negative water potentials than did control plants, even though a further decrease in the transpiration rate of antisense plants was detected compared with that of controls (Figure 4). A detailed list of the data obtained is given in Table 1.

NtAQP1 and Water Stress Resistance

NtAQP1 seems to contribute to water stress avoidance in tobacco. The treatment used in the drought experiments was relatively mild, reducing the soil water potential just from −0.01 to −0.07 MPa. These water stress conditions apparently were not strong enough to cause visible plant reactions. Thus, a more stringent water stress was used: irrigation with a polyethylene glycol (PEG) solution (138.4 g/L PEG 6000) with an osmotic potential of −0.35 MPa. PEG 6000 does not permeate roots; consequently, it imitates soil dehydration. Two to 3 hr after the addition of PEG, antisense plants started wilting, whereas controls remained visibly unaffected (Figure 5). This finding indicates that NtAQP1 function is required for resistance to extreme water depletion effects.

Table 1. Specific Hydraulic Conductivities, Transpiration Rates, and Water Potentials of Controls and Antisense Lines

| Line	Specific Root Hydraulic Conductivity (mmol m^{-2} s^{-1} MPa^{-1})	Specific Root Hydraulic Conductivity Freeze/Thaw (mmol m^{-2} s^{-1} MPa^{-1})	Transpiration Rate Watered (mmol m^{-2} s^{-1})	Transpiration Rate Drought (mmol m^{-2} s^{-1})	Ψ_{stem} Watered (MPa)	Ψ_{leaf} Watered (MPa)	Ψ_{stem} Drought (MPa)	Ψ_{leaf} Drought (MPa)
Control	26.5 ± 4.2 (n = 9)	114.0 ± 8.05 (n = 8)	2.99 ± 0.02 (n = 19)	1.82 ± 0.15 (n = 9)	−0.182 ± 0.04 (n = 8)	−0.297 ± 0.027 (n = 8)	−0.231 ± 0.018 (n = 8)	−0.397 ± 0.03 (n = 8)
NtAQP1-Antisense	11.9 ± 2.0 (n = 31)	109.5 ± 15.6 (n = 15)	2.77 ± 0.06 (n = 95)	1.23 ± 0.09 (n = 61)	−0.202 ± 0.008 (n = 20)	−0.32 ± 0.02 (n = 20)	−0.275 ± 0.014 (n = 20)	−0.437 ± 0.025 (n = 20)

DISCUSSION

NtAQP1 is the only plasma membrane intrinsic aquaporin from tobacco functionally characterized to date. In contrast to the model plant Arabidopsis, in which, as a result of the genomic sequencing approach, all aquaporin-like sequences are known, the number of genes in tobacco is uncertain. However, the classification of aquaporin-like proteins into the subfamilies PIP1, PIP2, TIP, NIP (nodulin-like intrinsic proteins), and SIP (small intrinsic proteins) can be applied to most plant species (http://mbclserver.rutgers.edu/CPGN/AquaporinWeb/Aquaporin.group.html). NtAQP1 belongs to the PIP1 family, and the degree of family sequence similarity to the PIP1s decreases in the order given above (i.e., from PIP2 to SIP).

Using unpublished sequence data (T. Mariani and M. Bots, personal communication), we were able to use probes of additional aquaporins belonging to the PIP1, PIP2, or TIP family for expression analyses of tobacco roots. These results are in agreement with those of studies of PIP1b antisense Arabidopsis plants (Grote et al., 1998) and show that only the closely related NtPIP1a is slightly affected by antisense NtAQP1 expression; other less similar mRNAs, like those from NtPIP2a and NtTIPa, accumulated to the level seen in control plants. Accordingly, the results obtained in the subsequent biophysical and physiological studies can account for the reduced expression of PIP1 family members and specify the function of these proteins.

We have investigated the cellular water permeability of root protoplasts, because NtAQP1 expression is prominent in the root, and the protein was detected in cells of the exodermis and endodermis as well as in the root cortex and xylem parenchyma (Otto and Kaldenhoff, 2000). Thus, cells containing NtAQP1 were accessible by the cell wall–degrading enzymes, and the resulting protoplasts were suitable for the water uptake test. The differences between root protoplast swelling rates clearly indicated that the classes of cells with relatively high water permeability (i.e., P$_{os}$ values from 16 to 32 μm/sec and from 32 to 64 μm/sec) were underrepresented in protoplast populations obtained from antisense

NtAQP1 plants. In contrast, cells showing water uptake rates from 8 to 16 μm/sec were abundant. Accordingly, our first conclusion is that NtAQP1 and possibly closely related PIP1 members increase the water permeability of cells expressing these aquaporins. A similar effect was observed in leaf protoplasts from antisense PIP1b Arabidopsis plants (Kaldenhoff et al., 1998). Although these plants developed a larger root system, possibly to compensate for the consequences of reduced cellular water permeability, tobacco did not show such morphological changes.
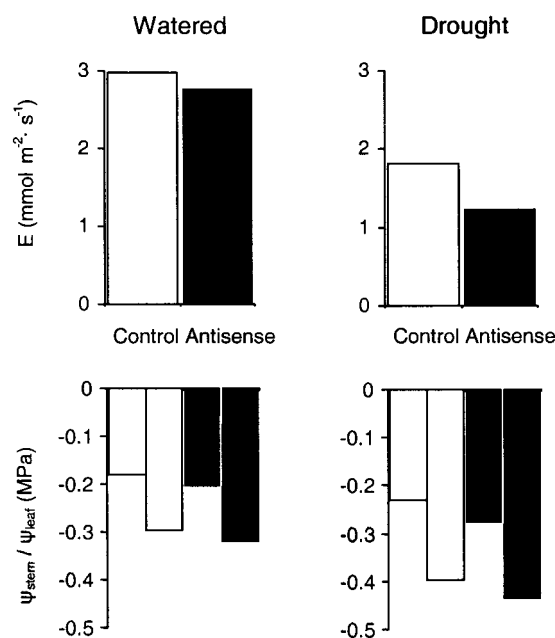
Figure 4. Data for Control and Antisense NtAQP1 Tobacco.

Transpiration rates (E; top), stem water potential (Ψ_{stem}; bottom left), and leaf water potential (Ψ_{leaf}; bottom right) of control (white bars) and antisense NtAQP1 (black bars) tobacco are shown. Plants were either watered or not watered for 1 week (drought). Complete data are given in Table 1.
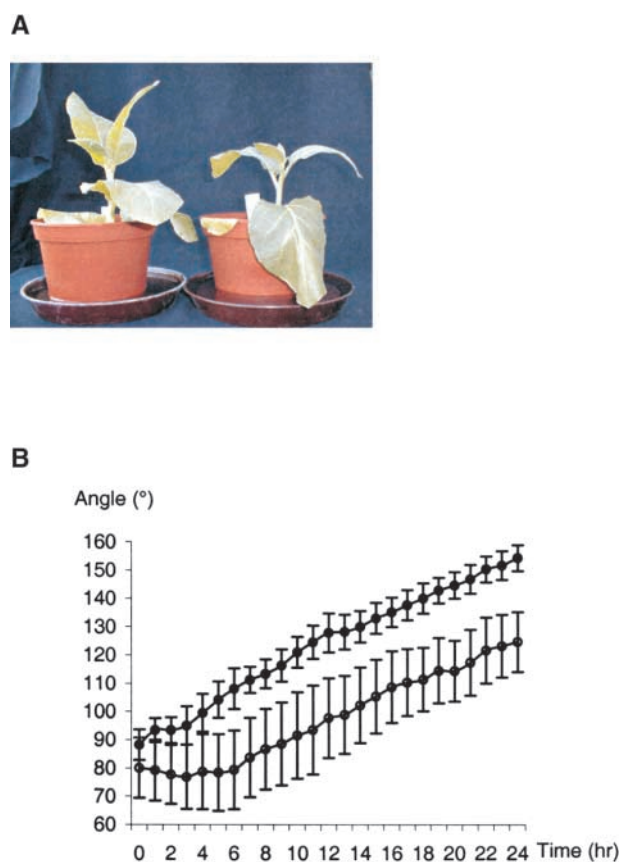
A

B

Figure 5. Water Stress Symptoms.

(A) Plants were watered with a PEG solution, inducing an osmotic pressure of −0.35 MPa, and photographed 2 hr later. Left, control plant; right, antisense NtAQP1–expressing plant.

(B) Average angles of leaves from plants treated with a PEG solution. Angles were measured in relation to the shoot axis for antisense NtAQP1 lines (closed circles; $n = 8$) and controls (open circles; $n = 6$).

Instead, physiological changes were observed. As a result of water permeability reductions in cells expressing NtAQP1 and NtPIP1s, the overall specific water conductivity of the root system was found to be reduced. This strong correlation between cellular water permeability and specific root hydraulic conductivity indicates the significance of the symplastic pathway with regard to vascular and long-distance water transport. It demonstrates our second conclusion, which is that cellular water transport is important and that purely apoplastic transport (e.g., in cell walls) is of minor impact in roots. Because the exact number of tobacco aquaporin genes and their pattern of expression still are unknown, evaluation of the precise relationship between the apoplastic and symplastic pathways remains

difficult. However, we conclude, on the basis of the hydraulic conductivity data presented here, that the ratio is at least 1:3. It would be shifted in favor of the symplastic pathway if other aquaporins that were not impaired by the antisense expression also contributed to cellular root water permeability.

As a second physiological response, caused directly or indirectly by signals generated by reduced aquaporin expression, antisense NtAQP1 tobacco changed the factors that alter water mobility. The plants decreased water potential and limited further water loss by reducing transpiration. In agreement with this interpretation is the observation that antisense NtAQP1 lines were less resistant to the addition of PEG. This treatment strongly decreased the soil water potential and induced strong water stress. The tobacco lines with reduced PIP1 aquaporin expression wilted earlier, possibly because critical tissue water potential and turgor pressure were reached much sooner. A correlation between water permeability changes in leaf cells (e.g., those in the stomatal cavity) and the early-wilting phenomenon under water stress is not apparent, because antisense lines responded to an appropriate stimulation of stomatal opening with the same transpiration kinetics as controls (data not shown). Accordingly, our third conclusion is that expression of NtAQP1 or a related PIP1 aquaporin contributes to water stress resistance.

In fact, for tobacco plants that were grown in well-watered greenhouse conditions, the PIP1 aquaporins did not seem to be important for water uptake or management. However, if increased hydraulic resistance caused by a lack of NtAQP1-like aquaporins combines with low soil water potential, antisense plants are not able to maintain turgor above the wilting point. They appear less drought tolerant. Thus, if water uptake becomes limiting, the expression of PIP1 aquaporins will enhance cellular water permeability, increase root hydraulic conductivity, relieve osmotic pumps, and support the survival of dry periods. Together, the data presented here provide an example of the function and physiological significance of aquaporins in plants. In addition, the importance of the symplastic water transport pathway was evaluated, and a new mechanism for water stress resistance in plants was described.

METHODS

Antisense Construct and Transformation

The NtAQP1 1.2-kb full-length cDNA was cloned in an inverse orientation into the binary vector pGPTV (11 kb) 3′ to a 35S promoter of *Cauliflower mosaic virus* and 5′ to a nopaline synthetase terminus. The resulting plant expression vector (pNtAQP1AS) was transformed into *Escherichia coli* TOP10F′. *Agrobacterium tumefaciens* strain LBA 4404 was transformed with pNtAQP1AS by triparental mating. Transformation and regeneration of transgenic tobacco (*Nicotiana*

tabacum) was performed according to the protocol provided by Gallois and Marinho (1995) with the exception that 500 mg/L carbenicillin was substituted for cefotaxime.

RNA Extraction and NtAQP1 Expression

Total RNA was isolated from tobacco roots using the Qiagen RNeasy Plant Mini Kit (Valencia, CA). Ten micrograms of total RNA from different preparations were size-fractionated on a 1% denaturing agarose-formaldehyde gel and transferred to a nylon membrane (Hybond N; Amersham Pharmacia) by capillary blotting. The nucleic acids were UV cross-linked and prehybridized in Roti-Hybri-Quick solution (Roth Chemikalien, Karlsruhe, Germany). Hybridization to ^{32}P-labeled probes and washing was performed at 68°C according to the manufacturer's guidelines. The air-dried membrane was exposed to x-ray retina film (XBD, Photochemische Werke, Berlin, Germany) with intensifying screens at -80°C, and the film was developed after an appropriate period of time. The resulting signals were quantified with Image Master VDS software (Pharmacia Biotech, Uppsala, Sweden). Values obtained for NtAQP1 were related to those of the tobacco 28S rRNA or actin after stripping and rehybridizing with a corresponding ^{32}P-labeled probe. The highest value obtained was set at 100%. All hybridizations were performed three times, and representative RNA gel blots are presented in the figures. In hybridizations with aquaporin-related probes, partial cDNAs corresponding to gene-specific regions were used as templates.

Preparation of Root Protoplasts

Approximately 20 root tips (1 to 1.5 cm long) were transferred to a Petri dish with 2 mL of enzyme solution, which consisted of 0.8% (w/v) cellulase (R 10; Onozuka, Duchefa, The Netherlands), 0.1% (w/v) pectolyase (Sigma), 0.5% BSA (Sigma), 0.5% polyvinylpyrrolidone, 1 mM CaCl$_2$, 10 mM KCl, 8 mM Mes, sorbitol to 300 mosmol, pH 5.5, and KOH. It was shaken at 100 rpm for 1.5 to 2 hr at 30°C in a rotary shaker in the dark and then filtered through 70-μm mesh. The material was rinsed with root isotonic solution (1 mM CaCl$_2$, 10 mM KCl, sorbitol to 300 mosmol, pH 5.5, and KOH). After 12 min of centrifugation at 100*g* at 20°C, the supernatant was removed, and protoplasts were resuspended in the remaining solution and used for the water uptake measurements.

Protoplast Water Uptake Measurements

The experimental chamber consisted of a straight groove in a lucite block with a central hole. Cells were assayed during a continuous solution flow (5 mL/min) provided by connected reservoirs of isoosmotic and hypoosmotic solution. A cover glass formed the bottom of the chamber. It was cleaned with ethanol, fixed below the hole with silicon grease, treated with poly-L-Lys (0.1%; Sigma) to immobilize the cells, and rinsed with isoosmotic solution. A drop of protoplast suspension was placed in the hole and left without any manipulation for 5 min. After covering the chamber with a microscope cover glass, it was rinsed with isotonic buffer. Protoplast volume was monitored at room temperature with a microscope video system (2 min, one frame/sec) directly before and after switching to hypoosmotic solution (180 mosmol). Care was taken that the distribution of cell sizes in the investigated individual protoplast population was similar (i.e., average diameter of 25.0 μm for antisense protoplasts and 24.5 μm for

control protoplasts). Buffer osmolarity in the chamber was determined using a Wescor Vapro (Wescor, Logan, UT) vapor pressure osmometer.

Determination of Osmotic Water Permeability

Protoplast volume was extrapolated from the recorded areas of the spherical cells. Relative volume change was plotted against time. Osmotic water permeability was calculated from the slope of the linear range of the graph according to Zhang and Verkman (1991) with the changes introduced by Ramahaleo et al. (1999), in which the initial protoplast volume and the initial protoplast surface area were determined for every protoplast. The molar volume of water is given as 18 cm^3/mol.

Plant Data Assessment

The results were obtained equally in four different antisense NtAQP1 lines. With the exception of data from RNA gel blot experiments, the data are presented together because only negligible variations were detected between individual lines. A tobacco line transformed with a 35S–β-glucuronidase construct was used for comparison and is designated "control" throughout. In initial experiments, it was found that the relevant plant responses were identical to those of untransformed tobacco (data not shown).

Plant Growth Conditions

Unless stated otherwise, plants were grown in normal greenhouse conditions in a 12-hr day/night cycle at 22°C in soil with regular irrigation.

Measurement of Specific Root Hydraulic Conductivity

Root hydraulic conductivity was determined as described by Tyree et al. (1995). Shoots were excised from the roots, and water was perfused from the root stalk to soil. The perfusion pressure was increased linearly from 0 to 500 kPa at $\sim$8 kPa/sec while flow readings were taken every 2 sec. The slope of flow versus pressure was divided by the root surface area to compute the root hydraulic conductivity.

Water Stress Experiments

A moderate water stress was induced by cultivation without irrigation for 1 week. Water content in the substrate was determined gravimetrically and related to soil water potential, which was assessed previously using the substrate pressure plate method (Richards et al., 1956).

Transpiration

Transpiration rate was assessed by gas exchange measurements from all leaves except two. Measurements were taken from 10:30 AM to 5:00 PM (air temperature, 34.7 $\pm$ 0.09°C; RH, 35.0 $\pm$ 0.24%; PPFD, 1462 $\pm$ 9.9 μmol·m^{-2}·sec^{-1}) using an open-system ADC-LCA3 infrared gas analyzer equipped with a Parkinson Leaf Chamber (Analytical Development Company, Hoddesdon, UK).

Water Potential

Leaf water potential and stem water potential were measured by the pressure chamber technique (Steudle, 2001) in leaves using a Scholander-Hammel bomb (Soil-moisture Equipment Corp., Santa Barbara, CA). Measurement of stem water potential was performed according to Shackel and Turner (2000). Two leaves per plant were wrapped 4 hr in advance of the measurement.

Accession Numbers

The accession numbers for the proteins described in this article are AJ001416 (NtAQP1), AF440271 (NtPIP1a), AF440272 (NtPIP2a), and AJ237751 (NtTIPa).

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